1979

Three studies on cold acclimation in woody plants.

Leslie Ann Alexander
University of Massachusetts Amherst

THREE STUDIES ON COLD ACCLIMATION IN WOODY PLANTS

A THESIS PRESENTED

BY

LESLIE ANN ALEXANDER

Submitted to the Graduate School of the University of Massachusetts in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

February 1979

Department of Plant and Soil Sciences

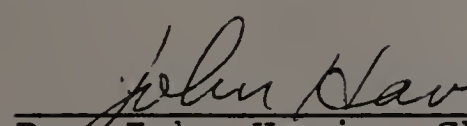
THREE STUDIES ON COLD ACCLIMATION

IN WOODY PLANTS

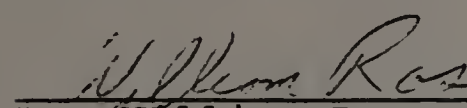
BY

LESLIE ANN ALEXANDER

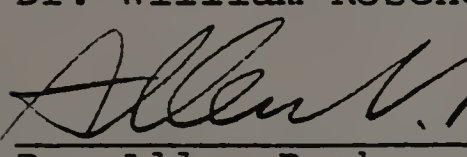
Approved as to style and content by:



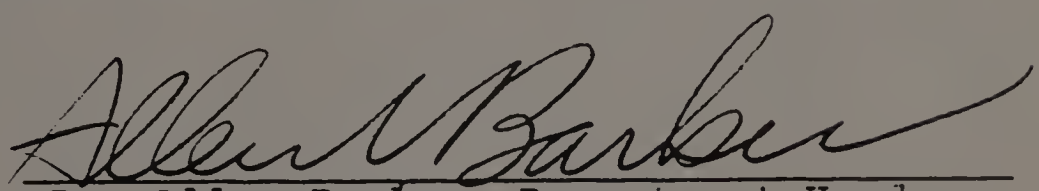
Dr. John Havis, Chairperson of Committee



Dr. William Roseneau, Member



Dr. Allen Barker, Member



Dr. Allen Barker, Department Head
Department of Plant and Soil Sciences

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The cold hardiness of woody plants is a major concern to nurserymen and orchardists in the north temperate zone. Cold temperature is one of the most limiting factors to plant growth and production. The stresses which are brought about by early fall and late spring frosts, rapid changes in temperature, and low mid-winter temperatures cause a variety of injuries to plant tissues. Some of these are death of flower buds, dessication of evergreens, death of vegetative shoots, and frost-splitting of trees. Cold hardiness is the ability of a plant to withstand cold stress and to avoid injury. Research in the field of plant cold hardiness seeks to discover how freezing temperatures kill plant tissues, and how some plants are able to acclimate and thus avoid freezing injury.

Freezing plant cells results in ice formation either within the cell or outside the cell wall (1). Intracellular freezing disrupts the integrity of the cellular membranes. Enzymes which are released destroy the already injured cells. Intracellular freezing occurs in plants which are incapable of acclimating but which have not attained an acclimated state. Extracellular ice formation occurs outside of the cell walls, between the cells (11). Hardy plants are those capable of surviving extracellular ice formation. Numerous hardy woody species can survive extracellular freezing at the

temperature of liquid nitrogen (-196°C) when fully acclimated (17, 18).

In hardy plants, ice formation begins outside the cell wall after a few degress of supercooling (1). Due to an increase in permeability during acclimation, the protoplast and cell membranes offer little resistance to the free passage of water to extracellular spaces. This allows the unbound water to freeze outside the cell (11). Cells which tolerate extracellular ice formation are in effect tolerating drought stresses, as much of the free cellular water has been removed to extracellular spaces. Research has demonstrated that among varieties of a given species, plants which tolerate dissication stress exhibit greater cold hardiness (3, 19).

Even hardy plants may suffer freezing damage. Although the exact mechanisms responsible for the death of hardy cells due to freezing are unknown, many hypotheses currently associate dehydration with the death of the cells. These theories postulate the denaturation of cellular proteins as a cause of cellular death. Levitt (11) has shown that damage occurs to the membranes of frost-injured cells. Weiser (23) proposes that when the "vital water" which is essential for the integrity of protoplasmic constituents freezes, the cell dies. This vital water is that which serves as a configurational component of proteins and nucleic acids.

Two factors determine the ability of a plant to survive cold stress. These are the genetic capacity of the species to withstand freezing temperatures and the conditioning or expression of that genetic capacity caused by environmental cues. Research has demonstrated that the environment controls the cold acclimation process by which hardy plants in a dehardened state acquire the capacity to resist cold injury. The gradual shortening of photoperiod in late summer and early fall appears to trigger the first stage of the acclimation process (16, 23). During this period growth stops, and changes in the plant metabolism occur. Total sugar and anthocyanin content have been shown to increase in proportion to cold hardiness (12, 15), while permeability of the cellular membranes increases (17) allowing cellular water to be transferred to extracellular spaces. Changes have also been noted in protein (21) and lipid contents (13) during this stage. The levels of auxins and gibberellins decrease and abscisic acid levels increase during the first phase of the acclimation process (7). Low temperatures induce the second stage of acclimation (6, 8, 9). The first frost may trigger this response (22). It has been proposed that during this stage further changes in the configuration of temperature-sensitive proteins occur increasing cellular resistance to cold temperature (1). Tissue hydration decreases and metabolic activity ceases (11).

Hormone-like factors induced by short days (SD) have been found in several species (4, 20). Studies have revealed that leaves exposed to shortening photoperiods are the source of translocatable cold-hardiness promoting factors (4), while leaves exposed to long days (LD) may produce factors which inhibit acclimation (9).

In a study of the effect of daylength on acclimation, exposure of leaves to artifically extended photoperiods during the fall inhibited cold acclimation (9). This might suggest that the LD leaf is the source of cold hardiness inhibitors. However, removal of the leaves did not produce the same degree of cold hardiness as in the control plants which were exposed to natural photoperiods. Therefore, exposure of leaves to SD photoperiods is necessary for full cold acclimation. A study by Howell and Weiser (6) further supports the hypothesis that SD exposure is essential to the first stages of cold acclimation. Removal of leaves during the first five weeks of SD exposure severely limited the acquisition of cold hardiness, while removal after the first five weeks had no significant effect on acclimation.

Grafting studies on diverse climatic races of a plant species have demonstrated that the hardiness promoting factors from the leaves of one genotype can enhance cold acclimation in another less hardy genotype. This suggests that the factors involved may not be genotypically specific.

Thus if the hardiness promoters can be isolated and identified, chemical programming of cold hardiness might be possible in plant species which have the inherent capacity to acclimate, but which do not acclimate in time to survive certain climates.

There has been little research on the cold acclimation process in roots. The main reason for this lack of research is the difficulty of studying the root in its environment. However, investigators have demonstrated that root hardiness is considerably less than that of the aerial portions of the same plants (2, 14). The killing temperatures of roots of many hardy woody ornamentals has been determined (5). One study which has examined the acclimation process in roots was carried out by Johnson and Havis (10). This study determined the importance of daylength and temperature on cold acclimation of roots of two plant species. They found that exposing the plants to LD photoperiods in the fall inhibited root hardiness. In addition, warm temperatures (above 15° C) reduced root cold acclimation under natural fall photoperiods. It is apparent from these results that short days and low temperatures are as necessary for cold acclimation of roots as for that of the aerial portions of plants.

Cold injury of roots is a serious problem in the overwintering of plants in nursery containers. Container

production of nursery crops has greatly expanded and with it the need for more information on the acclimation process and cold tolerance of roots.

PART I

Effects of Defoliation on Cold Acclimation in *Rhododendron* PJM Hybrids and *Cotoneaster horizontalis* Decne.

Additional Index Words: Winter-hardiness, Freeze-injury.

Abstract. The effects of manual defoliation of *Cotoneaster horizontalis* Decne., and *Rhododendron* PJM Hybrids at various time periods during the fall were measured. Defoliation of the evergreen *Rhododendron* at any time during the fall prevented acclimation throughout the plant; early defoliation of the deciduous *Cotoneaster horizontalis* interfered with the acclimation process in both branch and root sections.

Acclimation of woody plants to cold temperatures in late summer and early fall is stimulated by several environmental factors, notably light and temperature. Results from several controlled environmental studies have shown that the first stage of acclimation in most plants is a photoperiodic response to the shortening day length which is perceived by leaves (6, 7, 8, 10).

Many factors are involved in the short-day (SD) phase of the acclimation process. This stage of acclimation is dependent upon active metabolic processes, which are inhibited by low temperatures (1, 15, 18). In addition, growth cessation is a prerequisite to cold acclimation (1, 8). Short days are believed to be primarily responsible for

this cessation in woody plants (13, 18). Although active growth must stop, Irving and Lanphear (6) have shown that bud dormancy or rest is not necessary prior to cold hardiness development. Williams et al. (15) have shown that phytochrome mediates the SD enhancement of cold acclimation.

Further studies on the acclimation process have suggested the existence of hormone-like promoters and inhibitors involved in the hardening process (2, 6, 8, 15). Irving and Lanphear (7) found the long-day (LD) leaf a source of cold hardiness inhibitors. Under natural fall temperature, the removal of leaves from plants experiencing LD exposure produced significant hardiness as compared to the undefoliated plants. In addition, removal of leaves from plants exposed to LD and a lower temperature of 5°C brought about an accelerated rate of hardening. Steponkus and Lanphear (12), studying the light stimulation of cold acclimation, suggested that SD stimuli result in the production of a translocatable cold acclimation promoter. Their experiments indicate that movement of the promoter is initially acropetal and later in the acclimation process the flow is less restricted. Transport of the promoter substance is through the phloem (1, 12), and a stimulus of some leaves on a plant can limit the SD promoting effect of leaves on the same plant (7).

To study further the role of leaves in the acclimation process, the effects of defoliation on the development of plant hardiness were studied in an evergreen and a deciduous plant.

MATERIALS AND METHODS

Plants of Cotoneaster horizontalis, one year from cuttings, and PJM Hybrids, two years from cuttings, were grown in 15 cm containers with peat:sand (1:1 v/v) under natural environment conditions until November 8, 1976. At this time they were moved into a polyethylene-covered storage house where the minimum temperature was 0°. On September 15, and October 15, 36 plants of each species were manually defoliated, and meristems removed. New leaves were removed as they appeared. On November 15, 24 plants from each species were similarly defoliated.

Stem and root hardiness determinations were made on defoliated and control plants in November and December. Uniform twigs 3-4 cm in length, excised 2 cm from the tip and 3-4 cm of woody root of 1 mm diameter were tested for hardiness. Three plants from each species were used for test material. The sections were frozen at several test temperatures in a methanol bath at a rate of 2.5°C per hour. The tissue remained at the temperature to be tested for 1 hour, then was removed and allowed to thaw at 1°.

A modified ninhydrin test as described by Weist et al. (16) was used to evaluate injury. In addition, visual examination of tissue injury was made as described by Stergios and Howell (13).

In addition to the tests on excised tissues, roots of intact plants of both species, defoliated and control groups, were frozen at various temperatures in November, December and January according to the method described by Havis (4). Four plants were studied at each test temperature. After thawing, the plants were removed to the storage house. The plants were transferred to a warm greenhouse in mid-January and evaluated for injury in February.

RESULTS AND DISCUSSION

Results from the November 3 ninhydrin test (Table 1) indicate that the September defoliation of Cotoneaster interfered with the cold acclimation process in both the root and the branch sections. On December 8 the plants defoliated in September and October had less hardy stems and roots than the control plants. Both branch and root sections of the plants defoliated in November were as hardy as the corresponding sections of the control plants.

The results from freezing the root system of the intact Cotoneaster plants are given in Table 2. Roots of plants defoliated in September were less hardy than control

Table 1. Killing temperature (°C) of Cotoneaster horizontalis branch and root sections based on ninhydrin tests.

Test date	Treatment	Branch	Root
November 3			
	Control	-23	-6.7
	Defoliated September	-15	-4.0
December 8			
	Control	-23	-9.4
	Defoliated September	-15	-6.7
	Defoliated October	-20	-6.7
	Defoliated November	-26	-9.4

Table 2. Injury from freezing roots of intact Cotoneaster horizontalis plants on whole plant survival as observed February 7, 1977.

Test date	Treatment	Freezing temp (°C) 2	-4	-6.7	-9.4	-12
November 3						
	Control	0[z]	0	0	1	2
	Defoliated September	0	2	2	2	2
December 2						
	Control	0	0	0	0	2
	Defoliated September	0	2	2	2	2
	Defoliated October	0	0	0	2	2
	Defoliated November	0	0	0	1	2
January 5						
	Control	0	0	0	1	2
	Defoliated October	0	0	0	2	2
	Defoliated November	0	0	0	1	2

[z] Degree of freezing injury is represented by numerals: 0, normal; 1, plants injured; 2, all plants dead.

plants at all test dates. The roots of plants defoliated in October were much harder than plants defoliated in September and a few degrees less hardy than the control plants. The roots of plants defoliated in November appeared to be only slightly less hardy than roots of the control plants when tested in December, and results of the January test indicate that at this date, the roots of the November-defoliated plants are as hardy as the non-defoliated control plants.

Lower stems of the September defoliated Rhododendron were killed by a natural freeze of -6.7°C on November 2, 1976, before the plants were placed in storage. Results from the ninhydrin test on November 3 indicated that the roots of plants defoliated in October were killed at -18° while the roots of the control plants were injured at -23°.

Table 3 shows the results of freezing the roots of intact Rhododendron plants on November 3 and December 2, 1976. Roots of all plants defoliated in October were dead in February. Roots of plants defoliated November 15 were killed at -18° on December 2. These latter plants were sampled from the same population of non-defoliated control plants which on November 3 survived from -23° exposure.

An interesting comparison can be made of the ninhydrin and intact plant methods by comparing the results of the November and December test dates on Cotoneaster roots in

Table 3. Injury from freezing roots of intact Rhododendron PJM Hybrids plants based on whole plant survival as observed February 7, 1977.

Test date	Treatment	Freezing temp (C) 2	-12	-18	-23	-29
November 3						
	Control	0[z]	0	0	0	2
	Defoliated October	2	2	2	2	2
December 2						
	Control	0	0	0	0	2
	Defoliated November	0	1	2	2	2

[z] Degree of freezing injury is represented by numerals: 0, normal; 1, plants injured; 2, all plants dead.

Tables 1 and 2. The intact plant method gave lower root killing temperature than did the ninhydrin method in 3 cases, and the 2 methods gave the same results in 2 cases. The ninhydrin method indicated an increase in root hardiness between November 3 and December 2, but the intact plant method did not. The intact plant method may give the more accurate results since the results for this test are based on the response of the entire plant, while the ninhydrin method relys on excised root tissue. In order to obtain agreement between the two methods, the exact sections of root tissue necessary for plant survival must be chosen. This critical portion has not been fully determined. Studer et al. (14) have studied root hardiness of young and mature root tissue in a number of plant species. They found that young roots are more susceptible to injury and death due to low temperature than the mature roots. However, these young roots may not be essential for the survival of the whole plant. More studies are necessary in order to determine the exact root sections which are critical for whole plant survival.

The results of the studies on Cotoneaster horizontalis Decne indicate that defoliation of plants before the middle of November interfered with acclimation in both branch and root sections of the plants.

The results from the study on the evergreen Rhododendron PJM Hybrids indicate that defoliation in the fall prevents or restricts the acclimation process. In addition, defoliation in the fall may cause a loss of hardiness in plants which have started to acclimate. Thus the leaves of this species may be required not only to receive the photoperiodic stimuli necessary to trigger the first stage of acclimation, but also to maintain hardiness once the plant has started to acclimate.

Fuchigami et al. (3) reported that vegetative maturity is necessary before leaves can be removed from overwintering deciduous plants. During the fall the physiological changes due to environmental influences are essential if the plants are to acclimate. Their study suggests that the leaves are essential to perceive the shortening day length. This stimulus induces the plant to attain a metabolic level associated with vegetative maturity. The average date that a species reaches maturity differs from year to year due to the variations of the environmental stimuli. In their study on red-osier dogwood, Fuchigami et al. (2) found maturity to occur in late September while natural defoliation did not occur until late October. Defoliation of plants before the maturity date produced injury and death during winter storage, while plants defoliated after that date were uninjured.

Other studies on defoliation agree with the necessity of leaves during the first stage of acclimation. Hurst et al. (5) found that leaves removed or completely covered on Cornus stolonifera Michx. during a controlled environmental study markedly interfered with the development of cold resistance in the bark tissue. Their study indicated the necessity of the leaves to perceive the SD photoperiod for 7 to 14 days to induce cold hardening. Fuchigami et al. (1) removed leaves from C. stolonifera at various times over a 12-week acclimation period in a growth chamber and found that defoliation during the first 5 weeks interfered with the cold acclimation of the stems.

My results show that Cotoneaster responds to defoliation much like other deciduous plants, and that defoliation affects acclimation of roots as well as stems. In addition, I am not aware of any previous defoliation studies on evergreen. These studies suggest that defoliation may be even more harmful to evergreens than to deciduous plants.

PART II

A Comparison of the Cold Acclimation in an Evergreen and a Deciduous Azalea.

Additional index words: Rhododendron, Winter-hardiness, Freeze-injury.

Abstract. Cold hardiness of branch, lower stem, and root sections of the two cultivars were determined after artificial acclimation. After 53 days of acclimation, all parts of the deciduous azalea "Homebush" were hardier than the corresponding sections of the evergreen azalea "Mother's Day."

Killing of lower stem tissue, which frequently results in bark splitting, is an injury common to many species of evergreen azaleas. This lower stem injury often occurs following a fall with frosts and freezing temperatures occuring early in the season. This injury indicates that the plants have not had enough time to fully acclimate and avoid injury. However, injury of this type is not common in deciduous azaleas, which are generally more cold hardy than the evergreen varieties.

A delay in the acclimation process in the lower stem could account for the injury being localized in that area. Sakai (2) has studied cold hardiness in a variety of woody plants and found that the lower stem area is less hardy than

the upper parts of many plants.

Steponkus and Lanphear (3) studying the light stimulation of cold acclimation in Hedera helix found that hardiness-promoting substances moved through the phloem in an acropetal direction in the early stages of the acclimation process. As acclimation progressed, the promoters were found to move in a basipetal direction as well, and flow was then through the xylem as well as the phloem. Thus during the first weeks of acclimation, the induction of cold hardiness proceeded in phases throughout the plant, with the apex of the plant the first area to harden.

The following study was undertaken to follow the progression of cold acclimation in an evergreen azalea and a deciduous species.

MATERIALS AND METHODS

Plants, one year from cuttings, of an evergreen azalea "Mother's Day," a Kaempheri hybrid, and a deciduous azalea "Homebush," a Knap Hill hybrid, were grown in 15-cm azalea pots containing a 1:1 (v/v) mix of peat and sand. These plants were moved in mid-July, 1977, from an outdoor growing area into a growth chamber, where they received an 8-hour light period and steadily declining temperature. The temperature schedule was as follows: 7-10°C day, 4-7° night for 2 weeks, 4-7° day, 3° night for 4 weeks and 4-7° and 1°

night for 2 weeks. The plants were tested for cold hardiness at the beginning of the experiment and at the fourth and eighth weeks of the acclimation study. Samples from the branch, stem and root were used. Samples from the branch 3 cm in length were taken 3 cm from the tip. The lower stem sections were sampled from soil line up to 4 cm. Sections of woody roots 1 mm in diameter and 2-3 cm in length were excised. These roots are classified as secondary mature roots by Mityga and Lanphear (1). Four samples of each section from 3 plants were frozen at each of 4 test temperatures. The plant tissue was placed in a 50 ml plastic centrifuge tube, capped, and frozen in a circulating methanol bath, with a temp reduction of 2.5° C per hour. The tissue remained at the temperature to be tested for 1 hour and then was removed and allowed to thaw at 2°.

Two test methods to evaluate injury of the sampled tissue were used: a quantitative method, the modified ninhydrin test as described by Weist et al. (5), and a subjective test involving visual examination of the tissue for browning as described by Stergios and Howell (4).

RESULTS AND DISCUSSION

As the results in Fig. 1 indicate, differences in cold hardiness occurred between the evergreen and deciduous azaleas. After 53 days of acclimation in the growth chamber,

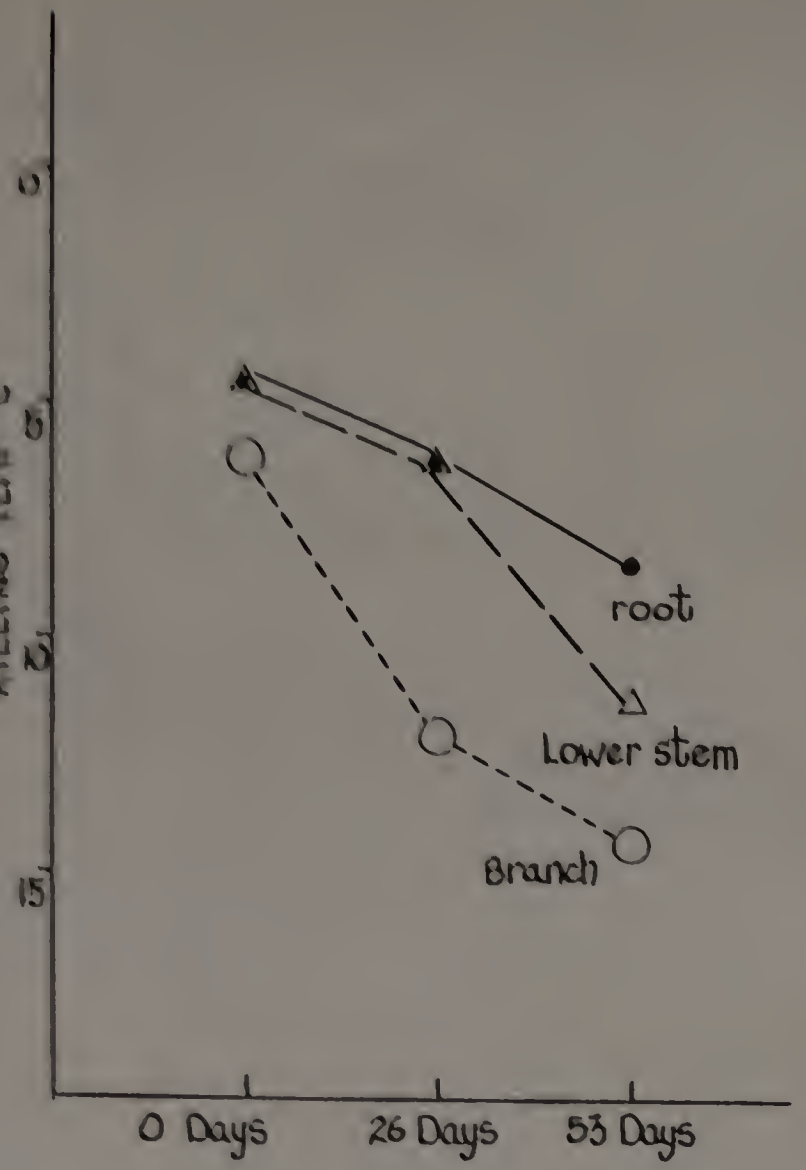
all parts--branch, lower stem and roots--of the deciduous azalea "Homebush" were hardier than the corresponding parts of the evergreen azalea "Mother's Day."

From these results, no preferential sequence of hardening can be seen in the two species of azaleas tested. Steponkus et al. (3) reported that hardiness promoters in Hedera helix moved in distinct stages during the acclimation process. Our study indicates that some degree of cold hardiness had developed in all plant parts in both species by the twenty-sixth day of the acclimating period. If a preferential apical flow of hardiness promoters exists in these plants, it may have occurred before the twenty-sixth day of the study, and, therefore, was not evident in our results. However, there was a difference in the acclimation patterns between the 2 species tested. The lower stem of the deciduous azalea "Homebush" appeared to acclimate at least as rapidly and to the same degree of cold hardiness as the upper branches. In contrast, the lower stems of the evergreen azalea "Mother's Day" were little hardier than the roots, and less hardy than the branches on all test dates. However, the slopes of the lines, which indicate the rate of acclimation, are similar. This suggests that the lower stem and branch followed a similar progression pattern.

Fig. 2 shows a comparison of the lower stem acclimation progression between the two species. Although they

were initially identical in hardiness, the lower stems of the deciduous azalea acclimated more rapidly than the evergreen and were hardier after both 26 and 53 days of acclimation.

These results suggest that differences in the rate and degree of acclimation may exist between deciduous and evergreen azaleas. Deciduous azaleas are generally more cold hardy than the evergreen species, and as the results from this study indicate, each section of the deciduous azalea was hardier than the corresponding sections of the evergreen plant at the termination of the experiment. The lower stems showed the largest differences. Obviously, various cultivars of evergreen azaleas differ in acclimation and ultimate cold hardiness. However, the slow rate of acclimation of evergreen azaleas, especially the lower stems, may account for the frequent incidence of lower bark injury seen in these species.



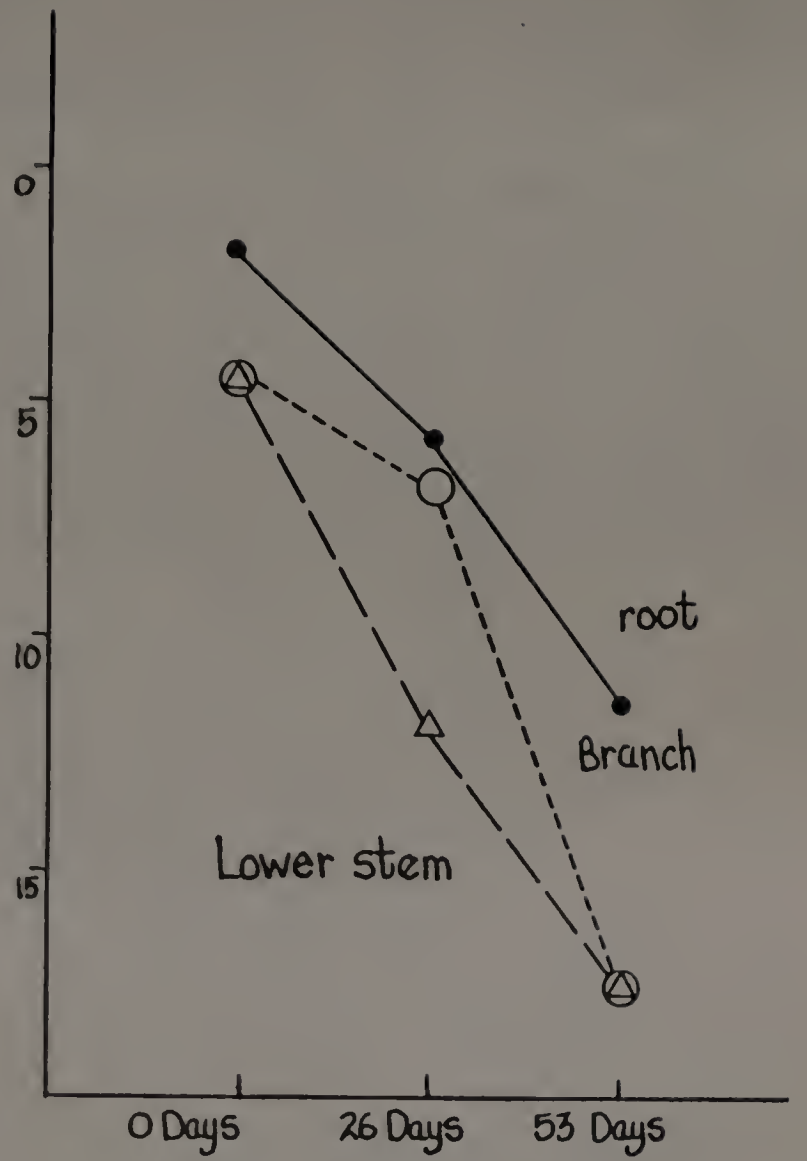


Fig. 1. Comparison of acclimation progression in root, lower stem and branch sections of the evergreen azalea "Mother's Day" and the deciduous azalea "Homebush."

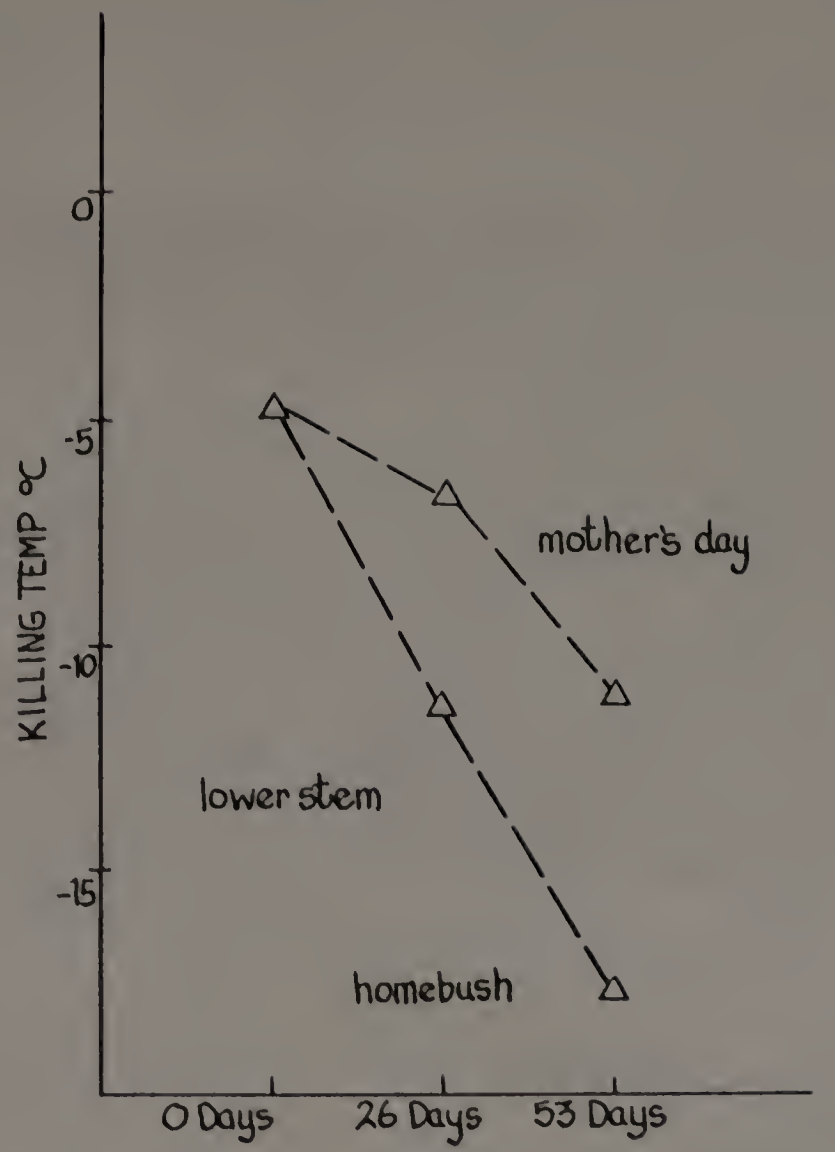


Fig. 2. Comparison of acclimation progression in lower stem sections of the evergreen azalea "Mother's Day" and the deciduous azalea "Homebush."

PART III

The Effects of Soil Temperature on Cold Acclimation of an Evergreen Azalea.

Additional index words: Rhododendron, Winter-hardiness, Freeze-injury.

Abstract. The effects of warm and cool root environments on the cold acclimation progression of branch, lower stem and root sections were measured. The warm root environment interfered with the acclimation process in all plant segments, with the roots and lower stem areas exhibiting the most interference.

Cold acclimation in woody plants is a physiological process brought about by a complex of environmental factors. It is generally agreed that acclimation proceeds in 2 phases. The progressive shortening of days in late summer, perceived by the leaves, is the stimulus for the first phase (5, 7, 8). The second stage is mediated by low temperatures (4, 8) and usually begins at the time of the first frost. Through the integration of these stimuli, the plant becomes acclimated.

Not all plants acclimate at the same time, and many hardy woody species are injured by early autumn freezes due to their tendency to acclimate slowly (3). Most of the plants which exhibit this problem are plants which are native

to areas of milder climates. These plants may possess the ability to harden and withstand the normal winter conditions of the northern temperate zones, if during their acclimation period the environmental conditions are characterized by gradually decreasing temperatures. However, they tend to sustain cold injury if the fall temperatures are warmer than usual, followed by a sudden cold period. One example of such injury is lower stem bark splitting of evergreen azaleas (3). Creech and Hawley (2) found that a 4" mulch around azaleas in the fall kept the soil warm, delayed the development of fall color and increased the amount of winter injury, including bark splitting.

Gardner (6) reported that the base of the trunk of many fruit trees seems to reach maturity in early winter more slowly than the other tissue, due to the persistence of cambial activity in this region. This leads to localization of winter injury in the immature regions. Sakai (11) studying a variety of woody plants, found that the basal sections of the stems were less hardy than the upper sections of the plants.

The following study was carried out in an attempt to determine the environmental effects of root temperature on the progression of cold acclimation.

MATERIALS AND METHODS

Beginning September 1, 1977, plants of the evergreen azalea "Springtime," a Kaempheri hybrid, one year from cuttings, in a medium of peat:sand 1:1 (v/v) were placed in 15 cm water-tight plastic azalea pots. Thirty-six plants were placed in 2 temp controlled circulating baths. Half of them were kept under warm conditions, where the water bath was kept at a minimum temp of 21°C for the first half of September, 19° for the second half of September, and 16° for the remainder of the experiment. The other plants were kept under the cooler conditions of 7° for the first 2 weeks of September, 5° for the next 2 weeks, and 1° for the remainder of the experiment. The plants were kept in an outdoor container area under natural fall conditions until October 18. They were then placed in a polyethylene-covered storage house where the air was kept above freezing. Temperatures of the branch, lower stem and soil were monitored by thermisters through a specially designed switching box and recorded on Rustrack recorders. The 2 water baths and the air temperature were also recorded (Table 4).

Cold hardiness of branch, stem, and root were studied at 3-week intervals starting September 1, and continuing through December. Four samples of each section from 3 plants were frozen at each of 5 test temperatures. The plant material was placed in 50 ml plastic centrifuge tubes

Table 4. Temperature (°C) ranges of the two water baths and ambient air temperature of the storage house.

	Cold root environment	Warm root environment	Air
September 1-17	6-8	21-26	13-27
September 18-30	3-6	16-22	7-23
October	1-3	16-21	2-27
November	1-3	16-21	0-11
December	1-3	16-17	0-11

with caps and frozen in a circulating methanol bath with temperature reduction of 2.5°C per hour. The tissue remained at the test temperature for 1 hour, then was removed to thaw at 2°. Two test methods to evaluate injury were used, a quantitative method, the modified ninhydrin test as described by Weist et al. (15), and a subjective test involving visual observation of the tissue for cold injury as described by Stergios and Howell (13).

RESULTS AND DISCUSSION

The results of the 2 root environments on the progression of cold hardiness are illustrated in Fig. 3. Acclimation was inhibited in all parts of the plants in the warm root environment. The lower stems and roots exhibited the greatest differences in cold hardiness. On the final test date, December 20, the roots of plants in the cold environment were 7°C hardier than those in the warm environment, and the lower stem tissue was 9° hardier in the cold root environment.

Analysis of variance of the cold hardiness progressions of plant parts between the two groups indicated that the killing temperatures for branch and lower stem were significantly different at the 0.05 probability level. While the difference in the root killing temperature was significant at the 0.01 probability level.

In addition to the variations of cold hardiness of the plant parts, visual differences in the foliage were noted. The azaleas in the cold root environment developed fall color on the uppermost leaves during the second week in October, with color apparent on the lower leaves during the third week in October, and full fall color attained by the end of October. Only slight color change was noted on plants in the warm root environment, and this was not apparent until mid-November. The warm root plants did not attain full fall color during the course of the experiment.

The failure of the warm root azalea plants to develop fall color may further indicate that the warm root environment interfered with the full development of cold hardiness. Parker (10) reported a correlation between anthocyanin content and cold hardiness in Hedera helix. In addition, Van Huystee (14) has observed that red stem pigmentation in red-osier dogwood appeared to coincide with the development of cold resistance of the bark.

All parts of the cold root plants acclimated steadily during the study. The warm root treatment produced a pattern of cold acclimation in lower stems and roots quite different from the cold root treatment. In September and October, these tissues in the warm root plants were less hardy but acclimated to some extent. However, the warm root treatment appeared to prevent any further acclimation

of lower stems and roots during November and December. The upper branches continued to acclimate during this period.

One might speculate that lower stems and roots of evergreen azaleas go through two stages of acclimation, the latter of which require cool roots -- at least cooler than the minimum 16°C to which the warm root plants were exposed in this experiment.

It is obvious that cold acclimation of all plant parts was greater in plants with cold roots than in plants with warm roots. A particularly interesting result was that the lower stems maintained essentially the same hardiness as the roots and were less hardy than the branches throughout the test period in plants of both treatments. These latter results agree with acclimation studies of another cultivar of evergreen azalea, "Mother's Day" (1).

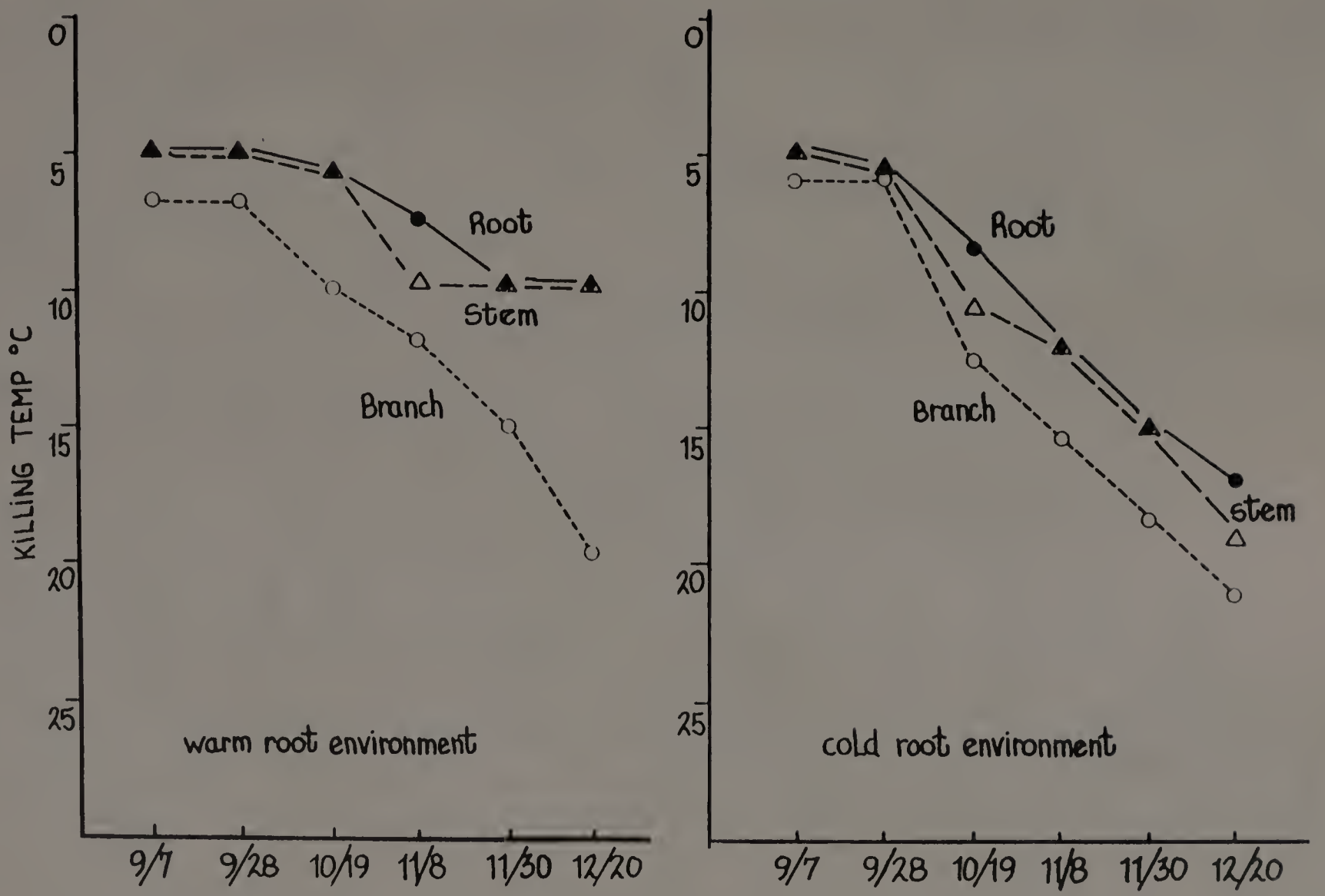


Fig. 3. Comparison of cold acclimation in sections of branch, lower stem and root of "Springtime" azalea plants subjected to warm and cold root environments.

BIBLIOGRAPHY

INTRODUCTION

1. Burke, M.J., L.V. Gusta, H.A. Quamme, C.J. Weiser, and P.H. Li. 1976. Freezing and injury in plants. Annual Rev. Plant Physiol. 27:507-528.

2. Chandler, W.H. 1913. The killing of plant tissue by low temperature. Missouri Agr. Expt. Sts. Res. Bul. 6.

3. Eguchi, T., A. Sakai, G. Usui, and T. Urhara. 1966. Studies of selection of frost hardy Cryptomeria L. Silvae Genetica. 15:84-89.

4. Fuchigami, L.H., D.R. Evert, and C.J. Weiser. 1971. A translocatable hardiness promoter. Plant Physiol. 42:1673-1679.

5. Havis, J.R. 1976. Root hardiness of woody ornamentals. Hort Science. 11 (4):385-386.

6. Howell, G.S. and C.J. Weiser. 1970. The environmental control of cold hardiness in apple. Plant Physiol. 45:390-394.

7. Hurst, C., T.C. Hall and C.J. Weiser. 1967. Reception of the light stimulus for cold acclimation in Cornus stolonifera Michx. Hort Science. 2:164-166.

8. Irving, R.M. and F.O. Lanphear. 1967. Environmental control of cold hardiness in woody plants. Plant Physiol. 42:1191-1196.

9. Irving, R.M. and F.O. Lanphear. 1967. The long day leaf as a source of cold hardiness inhibitors. Plant Physiol. 42:1384-1388.

10. Johnson, J.R. and J.R. Havis. 1977. Photoperiod and temperature effects on root cold acclimation. J. Amer. Soc. Hort. Sci. 102 (3):306-308.

11. Levitt, J. 1962. Responses of plants to environmental stress. Academic Press. N.Y.

12. Li, P., C.J. Weiser, and R. Van Huystee. 1965. Changes in metabolites of red-osier dogwood during cold acclimation. Proc. Am. Soc. Hort. Sci. 86:723-730.

13. Li, P., and C.J. Weiser. 1966. Evaluation of extraction and assay methods for nucleic acids from red-osier dogwood and RNA, DNA and protein changes during cold acclimation. Proc. Amer. Soc. Hort. Sci. 91:716-727.

14. Mityga, H.G. and F.O. Lanphear. 1971. Factors influencing the cold hardiness of Taxus cuspidata roots. J. Amer. Soc. Hort. Sci. 91:716-727.

15. Parker, J. 1962. Relationships among cold hardiness, water soluble protein, anthocyanins and free sugars in Hedera helix L. Plant Physiol. 809-813.

16. Perry, T.O., 1971. Dormancy of trees in winter. Science. 71:29-36.

17. Sakai, A. 1960. Survival of the twigs of woody plants at -196 C. Nature. 185:393-394.

18. Sakai, A. 1965. Survival of plant tissue at super-low temperatures III. Relation between effective prefreezing to temperatures and the degree of frost hardiness. Plant Physiol. 40:882-887.

19. Siminovitch, D. and R.D. Briggs. 1949. Studies on the chemistry of the living bark of black locust tree in relation to its frost hardiness. 1. Seasonal variations in protein content. Arch. Biochem. 23:8-17.

20. Steponkus, P.L. and F.O. Lanphear. 1967. Light stimulation of cold acclimation: Production of a translocatable promoter. Plant Physiol. 42:1673-1679.

21. Steponkus, P.L. 1969. Protein-sugar interactions during cold acclimation. Abstr. 87, 6th Annu. Meeting Soc. Crybiol., Cryobiol. 6:285.

22. Van Huystee, R., C.J. Weiser, and P.H. Li. 1967. Cold acclimation in Cornus stolonifera under natural and controlled photoperiod and temperature. Bot. Gaz. 128 (3-4): 200-205.

23. Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science. 169, 1269-1278.

PART I

1. Fuchigami, L.H., C.J. Weiser, and D.R. Evert. 1971. Induction of cold acclimation in Cornus stolonifera Michx. Plant Physiol. 47:98-103.

2. Fuchigami, L.H., C.J. Weiser, and D.R. Evert. 1971. Translocatable cold hardiness promoter. Plant Physiol. 47:164-167.

3. Fuchigami, L.H., M. Holtz, and C.J. Weiser. 1977. The relationship of vegetative maturity to rest development and spring bud break. J. Amer. Soc. Hort. Sci. 102:450-452.

4. Havis, J.R. 1976. Root hardiness of woody ornamentals. Hort Science. 11:385-386.

5. Hurst, C., T.C. Hall and C.J. Weiser. 1967. Reception of the light stimulus for cold acclimation in Cornus stolonifera Michx. Hort Science vol. 2:164-166.

6. Irving, M.R. and F.O. Lanphear. 1967. Environmental control of cold hardiness in woody plants. Plant Physiol. 42:1191-1196.

7. Irving, M.R. and F.O. Lanphear. 1967. The long day leaf as a source of cold hardiness inhibitors. Plant Physiol. 42:1384-1388.

8. Irving, M.R. and F.O. Lanphear. 1968. Regulation of cold hardiness in Acer negundo. Plant Physiol. 43:9-13.

9. Johnson, J.R. and J. Havis. 1977. Photoperiod and temperature effects on root cold acclimation. J. Amer. Soc. Hort. Science. 102:306-308.

10. Levitt, J. 1972. Responses of plants to environmental stresses. 697pp. Academic Press.

11. Perry, T.O. 1971. Dormancy of trees in winter. Science. 171:29-36.

12. Steponkus, P.L. and F.O. Lanphear. 1967. Light stimulation of cold acclimation: production of a translocatable promoter. Plant Physiol. 42:1673-1679.

13. Stergios, B.G. and G.S. Howell, Jr. 1973. Evaluation of viability tests for cold-stressed plants. J. Amer. Soc. Hort. Science. 98:325-330.

14. Studer, E.J., P.L. Steponkus, G.L. Good, and S.C. Weist. 1978. Root hardiness of container-grown ornamentals. Hort.Science. 13(2):172-174.

15. Williams, B.J., N.E. Pellett, and R.M. Klein. 1972. Phytochrome control of growth cessation and initiation of cold acclimation in selected woody plants. Plant Physiol. 50:262-265.

16. Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science. 169:1269-1278.

17. Weist, S.C., G.L. Good, and P.L. Steponkus. 1976. Evaluation of root viability following freezing by the release of ninhydrin-reactive compounds. Hort.Science. 11(3):197-199.

18. Van Huystee, R.B., C.J. Weiser, and P.H. Li. 1967. Cold acclimation in Cornus stolonifera under natural and controlled photoperiod and temperature. Bot. Gaz. 128(3-4):200-205.

PART II

1. Mityga, H.G., and F.O. Lanphear. 1971. Factors influencing the cold hardiness of Taxus cuspidata roots. J. Amer. Soc. Hort. Sci. 96:83-86.

2. Saki, A. 1967. Frost damage on basal stems in young trees. Contr. Inst. Low Temp. Sci. B 15:1-14.

3. Steponkus, P.L. and F.O. Lanphear. 1971. Light stimulation of cold acclimation : Production of a translocatable promoter. Plant Physiol. 42:1673-1679.

4. Stergios, B.G. and G.S. Howell. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98:325-330.

5. Weist, S.C., G.L. Good, and P.L. Steponkus. 1976. Evaluation of root viability following freezing of ninhydrin-reactive compounds. Hort.Science.11:197-199.

PART III

1. Alexander, L.A. Effects of defoliation on cold acclimation in an evergreen and a deciduous azalea. Part II of this Thesis.

2. Creech, J.L. 1959. Effects of mulching on growth and winter injury on evergreen azaleas. J. Amer. Soc. Hort. Sci. 75:650-657.

3. Flint, H.A. 1966. Seasonal hardening in trees and shrubs. Arnoldia. 20:57-60.

4. Fuchigami, L.H., C.J. Weiser, and D.R. Evert. 1970. Induction of cold acclimation in Cornus stolonifera Michx. Plant Physiol. 47:98-103.

5. Fuchigami, L.H., C.J. Weiser, and D.R. Evert. 1970. A translocatable cold hardiness promoter. Plant Physiol. 47:164-167.

6. Gardner, V.R., F.C. Bradford, and H.D. Hooker, Jr. 1939. The Fundamentals of Fruit Production. Second edition. McGraw-Hill, N.Y.

7. Hurst, C., T.C. Hall and C.J. Weiser. 1967. Reception of the light stimulus for cold acclimation in Cornus stolonifera Michx. Hort Science. 2:164-166.

8. Irving, R.M. and F.O. Lanphear. 1967. Environmental control of cold hardiness in woody plants. Plant Physiol. 42:1191-1196.

9. Mityga, H.G. and F.O. Lanphear. 1971. Factors influencing the cold hardiness of Taxus cuspidata roots. J. Amer. Soc. Hort. Sci. 96:83-86.

10. Parker, J. 1962. Relationships among cold hardiness, water soluble protein, anthocyanins and free sugars in Hedera helix. L. Plant Physiol. 809-813.

11. Sakai, A. 1967. Frost damage on basal stems in young trees. Contr. Inst. Low Temp Sci. B 15:1-14.

12. Steponkus, P.L. and F.O. Lanphear. 1971. Light stimulation of cold acclimation: production of a translocatable promoter. Plant Physiol. 42:1673-1679.

13. Stergios, B.G. and G.S. Howell. 1973. Evaluation of viability tests for cold stressed plants. J. Amer. Soc. Hort. Sci. 98:325-330.

14. Van Huystee, R.B. and C.J. Weiser, and P.H. Li. 1967. Cold acclimation under natural and controlled photoperiod and temperature. Bot. Gaz. 128:200-205.

15. Weist, S.C., G.L. Good, and P.L. Steponkus. 1976. Evaluation of root viability following freezing by the release of ninhydrin-reactive compounds. Hort.Science. 11:197-199.